

Stochastic Model of Maturation and Vesicular Exchange in Cellular Organelles

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ABSTRACT The dynamical organization of membrane-bound organelles along intracellular transport pathways relies on vesicular exchange between organelles and on biochemical maturation of the organelle content by specific enzymes. The relative importance of each mechanism in controlling organelle dynamics remains controversial, in particular for transport through the Golgi apparatus. Using a stochastic model, we show that full maturation of membrane-bound compartments can be seen as the stochastic escape from a steady-state in which export is dominated by vesicular exchange. We show that full maturation can contribute a significant fraction of the total out-flux for small organelles such as endosomes and Golgi cisternae.

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The hallmark of eukaryotic cells is their compartmentalization into specialized membrane-bound organelles that constantly exchange components with one another through the budding and fusion of small transport vesicles. Inter-organelle exchange is tightly regulated, leading to directional intracellular transport along well-defined pathways [1]. Regulation is permitted by molecular recognition during vesicle budding and fusion, and involves different kind of membrane-associated proteins such as coat proteins to create the vesicle, and tethers and SNAREs to control vesicle fusion [2]. This machinery is orchestrated by components defining the biochemical identity of the organelles' membrane such as members of the Rab GTPases family [3, 4]. The identity of the organelle membrane changes in time in a process called maturation. Rab5 positive early endosomes mature into Rab7 positive late endosomes over a time scale of order 10 min [5]. The sub-compartments of the Golgi apparatus called cisternae, which are dispersed throughout the cytoplasm in *Saccharomyces cerevisiae*, can be seen to mature from a *cis* to a *trans* identity in about 1 – 2 min [6, 7]. During the maturation process, the two membrane identities coexist in the organelle membrane, which sometimes show phase segregation [6]. In most animal and plant cells, Golgi cisternae are stacked together in a polarized way, with an entry (*cis*) face and an exit (*trans*) face, and whether Golgi transport occurs by inter-cisternal *vesicular exchange* or by full *cisternal maturation* is still highly controversial [8]. This question is of high physiological relevance considering the involvement of Golgi dysfunction in many pathologies, including Alzheimer and cancers [9, 10, 11, 12, 13]. We present a theoretical model that allows to precisely quantify whether progression along a model transport pathways predominantly occurs by vesicular exchange between organelles of fixed biochemical

identities or by the biochemical maturation of the organelles themselves.

Physical models of intracellular transport have been developed in recent years [14, 15, 16, 17]. These studies generally focus on steady-state properties, and the inherently stochastic nature of intracellular transport has been much less explored [18]. Stochasticity should however play an important role since the fusion/budding of a few tens of vesicles (of diameter $\sim 50 - 100$ nm), is enough to completely renew the membrane composition of an endosome or a Golgi cisterna (of area $\sim 0.2 - 0.5 \mu\text{m}^2$). This explains the strong fluctuation of the size and composition of early endosomes [5]. Our model establishes the importance of stochasticity in controlling the balance between maturation and exchange, and identifies the key control parameters as being the ratio of maturation to vesicle budding rates and the steady-state organelle size. In our model, sketched in Fig.1, we consider a membrane-bound compartment that receives a fixed influx J of vesicles of a given identity called A , corresponding to the early, or *cis* identity. A maturation process converts the compartment's membrane into a late, or *trans* identity B at a given maturation rate k_m . Finally, mature membrane components are exported out of the compartment by selective vesicle budding at a vesiculation rate K . We choose the simplest possible case where the maturation and budding rates are constant per unit area, and choose to express all sizes in terms of the size of transport vesicles, assumed the same for the in-flux and the out-flux. The compartment size and composition are entirely defined by the numbers N_A and N_B of A and B components in the system, or equivalently the total size $N = N_A + N_B$ and the fraction of mature (B) components $\phi = N_B/N$.

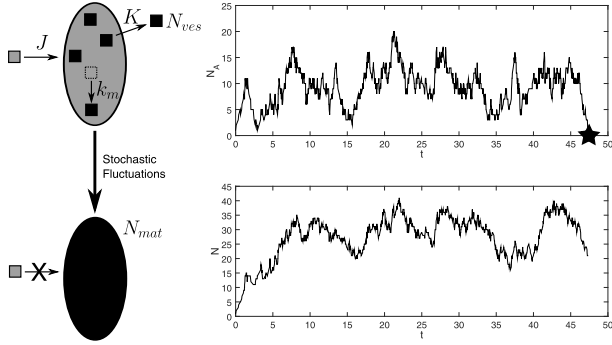


FIGURE 1 (Left) Sketch of the model: the compartment receives a constant in-flux J of immature (A - grey) components which mature into B (black) components at a rate k_m , and are exported by vesicular exchange at a rate K . Stochastic fluctuations lead to the full maturation and export of the entire compartment, after what a new compartment is created *de novo*. (Right) Typical time trace showing the fluctuations of the content in A component N_A (top) and compartment total size N (bottom) as a function of time until full maturation (black star).

At the mean-field level the temporal evolution of these quantities is given by

$$\dot{N}_A = J - k_m N_A \quad \dot{N}_B = k_m N_A - K N_B \quad (1)$$

which leads to the steady-state

$$N = \frac{J}{k_m(1 - \phi)} \quad \phi = \frac{k_m}{k_m + K} \quad (2)$$

The steady-state corresponds to *vesicular exchange*. The vesicular in-flux of immature components J is entirely converted into vesicular out-flux of mature components. In practice however, all the processes defining the exchange and maturation dynamics are stochastic, and one expects fluctuations around the steady-state, which will be comparatively more important for small compartments. Because of these fluctuations, the compartment will eventually reach the state where it is entirely composed of B components. Invoking the process of homotypic fusion, namely the propensity of a vesicle to fuse with a compartment with similar composition of Rabs, both in the endosomal network [5], and in the Golgi apparatus [19], we consider that an incoming A vesicle does not fuse with a compartment only composed of B components. The fully mature compartment thus becomes isolated from the in-flux, it exits the system as part of the out-flux, and a new compartment is created from scratch. This corresponds to the *cisternal maturation* mechanism of Golgi transport [8]. Our system being capable of showing both behaviors, the dominant transport mechanism must be obtained from a statistical analysis of the out-flux. We propose to compute the output parameter η defined as follows:

$$\eta = \frac{\langle N_{ves} \rangle}{\langle N_{ves} \rangle + \langle N_{mat} \rangle} \quad (3)$$

Where $\langle N_{ves} \rangle$ is the average number of type B vesicles emitted by the compartment before full maturation ($\phi = 1$), and

$\langle N_{mat} \rangle$ is the average size of the fully matured compartment. The out-flux is dominated by vesicular transport if $\eta \simeq 1$ and by full compartment maturation if $\eta \ll 1$.

We performed numerical simulations of the stochastic equivalent of Eq.(1), following the scheme described in the *Supporting Material*. The computed value of η for different values of the ratio k_m/K and J/K are shown in Fig.2 as a phase diagram. The two extreme mechanisms of *vesicular exchange* ($\eta \approx 0$) and *compartment maturation* ($\eta \approx 1$) are observed for extreme values of the parameters, namely large steady-state compartment size N for the former and high value of the steady-state fraction ϕ for the latter. However, the full picture is much richer, and a gradual transition between the two mechanisms is expected upon variation of the ratio of maturation to budding rates for intermediate compartment size.

To obtain an analytical approximation for the output parameter η , we first compute the mean first passage time to isolation. This can be done exactly because the dynamics of A components in the compartment is independent of the dynamics of the B components. The transition rates governing the evolution of $N_A(t)$ are:

$$N_A \xrightarrow{J} N_A + 1 \quad N_A \xrightarrow{k_m N_A} N_A - 1 \quad (4)$$

The average time τ_n needed to reach $N_A = 0$ starting from a state $N_A = n$ following this simple stochastic process satisfies the classical recursion law for mean first passage times [20] adapted to this system (see *Supporting Material*):

$$-1 = k_m n(\tau_{n-1} - \tau_n) + J(\tau_{n+1} - \tau_n) \quad (5)$$

An expression for the average time to full maturation starting from a newly created compartment, τ_1 , can be obtained by

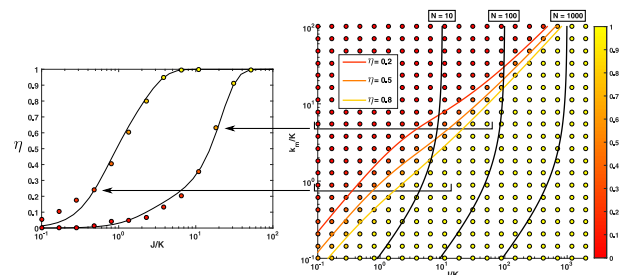


FIGURE 2 Simulation results. (Right) Phase diagram: the value of the output parameter η as a function of the in-flux J and maturation rate k_m , normalized by the budding rate K , illustrates the transition between the *vesicular exchange* ($\eta \simeq 1$) and *compartment maturation* ($\eta \simeq 0$) regimes. The black lines show constant values of the compartment size in the pseudo steady-state (Eq.(2)). The three colored lines represent constant values of η as given by the approximate analytical computation of Eq.(10). (Left) Cuts through the phase diagram varying the compartment size for two fixed pseudo steady-state compositions.

solving Eq.(5) recursively to obtain the expression:

$$\frac{\tau_{n+1} - \tau_n}{n!} = \left(\frac{k_m}{J}\right)^n \tau_1 - \frac{1}{J} \sum_{i=0}^{n-1} \frac{1}{(n-i)!} \left(\frac{k_m}{J}\right)^i \quad (6)$$

For $n \gg J/k_m$ Eq.(1) shows that the system evolves toward the stationary state, in a typical time of the order of $1/k_m$ independent of n , before full maturation. Therefore we expect that $\lim_{n \gg 1} (\tau_{n+1} - \tau_n)/n! = 0$, which leads to an explicit formula for τ_1 :

$$\tau_1 = \frac{e^{\frac{J}{k_m}} - 1}{J} = \frac{e^{N(1-\phi)} - 1}{k_m N(1-\phi)} \quad (7)$$

where N and ϕ are the steady-state average size and composition of the compartment (Eq.(2)).

To estimate the value of the output parameter η (Eq.(3)), we must compute the average number of vesicles emitted before compartment isolation $\langle N_{ves} \rangle$, and the average size of the fully matured compartment $\langle N_{mat} \rangle$. Calculating these quantities analytically is difficult, because it requires solving the two-dimensional isolation problem for N_A and N_B . An estimate of the size of the matured compartment is $\langle N_{mat} \rangle \simeq N\phi$. This amounts to saying that isolation occurs due to a temporary lack of incoming vesicles, while the number of B components retains its steady-state value due to a balance between maturation and vesicle secretion. An estimate of the amount of emitted vesicles before maturation can be obtained by supposing that the system spends most of its time undergoing small fluctuations around the steady-state, so that: $N_{ves} \approx K N \phi \tau_1 = e^{N(1-\phi)} - 1$. The fact that newly formed compartments are initially small and may reach full maturation without ever reaching the steady-state modifies this estimate. This can be crudely taken into account by considering that the initial compartment made of one vesicle can mature directly and become isolated in only one step. This corresponds to $N_{ves} = 0$ and $N_{mat} = 1$ and happens with the probability:

$$p_1 = \frac{k_m}{k_m + J} \quad (8)$$

Taking this into account, we obtain the following estimates:

$$\begin{aligned} N_{ves} &\approx (1 - p_1)(e^{N(1-\phi)} - 1) \\ N_{mat} &\approx p_1 + (1 - p_1)N\phi \end{aligned} \quad (9)$$

from which we get the approximate results:

$$\frac{\eta}{1 - \eta} = \frac{\frac{J}{k_m} \left(e^{\frac{J}{k_m}} - 1 \right)}{1 + \frac{J^2}{K k_m}} = \frac{N(1 - \phi) (e^{N(1-\phi)} - 1)}{1 + N^2 \phi (1 - \phi)} \quad (10)$$

This expression is compared to the results of the numerical simulations in Fig.2. The approximation is quite good for the entire range of η , except for small compartments (small value of J/K).

The highly dynamical nature of intracellular organization requires the exchange processes between organelles to be tightly regulated in order to yield robust directional flow of material through the cell. While the cell may to some extent be viewed as the steady-state of a complex, dynamical system, specific budding, fusion and maturation events, which are at the heart of its organization, are inherently stochastic processes. Stochastic fluctuations should thus play an important role in this organization, owing to the relatively small size of many cellular organelles. We have developed a stochastic dynamical model to study the interplay between maturation and exchange in intracellular trafficking. Our model reproduces the strong fluctuations of size and composition seen in early endosomes [5] (Fig.1b), and contains the two extreme exchange mechanisms at the heart of the Golgi transport controversy [8]; *vesicular exchange* and *compartment maturation* as asymptotic solutions. We identify full compartment maturation as a first passage time process. As a result, this mechanism only plays a dominant role for very small compartments or when the maturation rate is very large compared to the budding rate. However, it does amount to a sizable fraction of the total out-flux for intermediate compartment sizes and compositions (Fig.2). Regulation of the export mechanism could be very important for the transport of large cargoes that do not fit inside export vesicles. Our results suggest a possible mechanism for such regulation. The presence of a large cargo such as procollagen aggregates in Golgi cisternae [21, 22] could reduce the rate of vesicle secretion K , e.g. by mechanical means through an increase of membrane tension imposed by the distension of the cisternal membrane. This would favor full cisternal maturation and permits the progression of the large cargo through the Golgi stack.

SUPPORTING CITATIONS

Reference [23] appears in the Supporting Material.

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